

From Page No. 96 Book # 17041

Sequence of Tyro 10 (HPTK6) full length

> /home/sprite/vc/KessasDev/will/yeast/tyro/aa.new10  
> size: 6 cutters  
> length: 1637 (linear)

```
xhoI salI
paar7II
ecoRI hincII/hindII bsmI          ecoRII auaI          eco01091/draII
apoi auaI accI          bsmI          bsmI/mstII/sauI          bsmI/mstII/sauI          eco01091/draII
1 GAATTCCTGA GTCCGACCTG GACTTGAAGG AATGCCAAGA CATCTCTCCC CCACCCCTCT AGCCCTCCAGG CATCAGGAC TATCGGACCA GAGCCCTCTG
CTTAAGAGCT CAGCTCCACG CTGAACCTCC TTACGGTTCT CTACGACGGG GGTGGGGGAA TCGGGGCTCC CTATGCTCTG H G P E A L S
1
sphi
nspi
nspiII bsmII
201 CATCTTACTT CACTCTCTCT TTGCTGCGCA GTGGAGATCT TCACATCAAG GCACATTTTG ATCTCTGCGA GTCCCTCTAT CCGCTGCGCA TCGAGGACCG
CTAGAAATCA CCGACGCGAG AACACAGGCT CACTCTCTCT ACTGTACTTC CTGTAAAGAC TAGGACGCTT CACCGCGATA CGGACGCTCT ACCTCTCTGC
S L L L L L L L V A S G D A D M K G H P D P A K C R Y A L G M Q D R
8
bep1286
bmyI
bgiCI
banII
201 GACCATCCCA GACAGTGACA TCTCTCTCTT CAGCTCTCTG TCAGATTTCA CTGCGCCCGG CCACAGCAGG TTGAGAGCA GTACACGCGA TCGGCTCTG
CTGTATAGCT CTGTCTACTCT AGAGAGGAGG GTGCGAGGAG AGTCTTAGGT GACCGCGCGC GGTGTCTCTC AACCTCTCTT CACTCTCTCT ACCTCTCTGC
41 T I P D E D I S A S S S W E D S T A A R N E R L E S S D G D G A W
alwNI pfiNI bspHI          eco01091/draII
301 TGCCCTCCAG GGTCTCTGTT TCCCAAGGAG GAGGAGTACT TCGAGGTGCA TCTACAAACA CTGACCTCTG TGCTCTCTGT GCGGACCTAG CGAGCGCATG
ACCGGCGCTC CCAGCCACAA AGCGTCTCTC CTCTCTCATGA ACCTCACTCT AGATCTCTCT GAGCTGCGAC ACCGAGACCA CCGCTCTCTC CTCTCTCTAC
74 C P A G S V F P K E E E Y L Q V D L O R L H L V A L V G T Q C R N A
xmaI
smaI
ecoNI          cfr10I          auaI          nspBII          nspBII
401 CCGCGCGCTT GCGCAAGGAG TTCTCTCCGA GTACCGCGCT GCGTCTCTCT CCGGATGCTC CCGCTCTCTG GCGCTCTCTG GCGCTCTCTG GTACGAGCT
GCGCGCGCTC CCGCTCTCTC AAGAGCGCTT CAGTCTCTCA CCGTCTCTCT CCGGATGCTC CCGCTCTCTG GCGCTCTCTG CCGCTCTCTG CAGTCTCTCA
108 G G L G K E F R S Y R L R T S A D G R R W N G M K D R M G Q R V
xmaI
smaI
bgiJII
eco01091/draII
bep1286
bep120I
bmyI
banII
501 GATCTCAGC AATGAGGAG GTAGGAGG GTCTCTCTCT GAGCTCTCTG CCGCTCTCTG TCGCTCTCTG GCGCTCTCTG GCGCTCTCTG TCGCTCTCTG
CTAGAGCTCT TTACTCTCTG GACTCTCTCA CCGAGCTCTC CTGAGACCG GCGCTCTCTG CCGCTCTCTG CCGCTCTCTG CCGCTCTCTG CCGCTCTCTG
141 T S G M E D P E G V V L E D L O P F M V A R L V R F Y P R A D R V
sstI
sacI
bgiJII
bgiAI/nspHI
sc1116II
bep1286
bgiHKAI
bmyI
banII
601 ATGAGCTCT GTCTCTCTGT AGAGCTCTCT GTCTCTCTCT GAGGAGGAGT ACTCTCTCTT TACACCGCTT CTCTCTCTCT GAGCTCTCTT TATCTCTGAG
TACTCTCTCT CAGAGCGCGA TCTCTCTCTT CCGAGCGGAG GTCTCTCTCT TCGAGCGGAG ATCTCTCTCT GAGAGCGCTT CTCTCTCTCT TATCTCTCTT
174 H S V C L R V E L Y G C L W R D G L L E S Y T A P V G Q T H Y L E S A
scfI          pvuII
petI          nspBII
701 CCGTCTCTCT CAGAGCTCTC ACCTATGAGG GACATACCTT GCGCGGAGT GAGTATGAGG GTCTCTCTCT GCTCTCTCTG GCTCTCTCTG GCTCTCTCTG
GGCAGATGGA GTCTCTCTCT TCGATCTCTG CTCTCTCTCA CCGCGGAGT GCTCTCTCTG GCTCTCTCTG GCTCTCTCTG GCTCTCTCTG GCTCTCTCTG
206 V Y L N D S Y T D G H T V G G L Q Y G G L G Q L A D G V V G L D D
nscI/banII
haeI
nseI
alwNI          nseI          nseI          nseI
801 TTTCAGCGAG ACTCTCTCTG TCGCTCTCTG GCGAGCTCTT GAGTATGAGG GATGAGCGAA CCAGAGCTCT TCGAGTCTCT ATCTCTCTCT GCGAGCTCTG
GAACTCTCTT TCGCTCTCTG ACCTCTCTCT GCGAGCTCTT GAGTATGAGG GATGAGCGAA CCAGAGCTCT TCGAGTCTCT ATCTCTCTCT GCGAGCTCTG
241 F R E S Q E L R V M P G Y D V G W S H N S F S G Y V E N E F E
bgiJII
bep1286
bmyI
banII
901 TTTCAGCGAG TCGAGCTCTT CCGAGCTCTG CAGCTCTCTT GAGTATGAGG GATGAGCGAA CCAGAGCTCT TCGAGTCTCT ATCTCTCTCT GCGAGCTCTG
AACTCTCTCT ACTCTCTCTG GCGAGCTCTT GAGTATGAGG GATGAGCGAA CCAGAGCTCT TCGAGTCTCT ATCTCTCTCT GCGAGCTCTG
274 F D R L R A P Q A N Q V H C H N H E T L G A R L P G C V E C R F R R
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Date

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Date

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From Page No.           

1001 GTGCGCTG CATGCGCTG GAGGCGGAGC CCATGCGGCA CAACCTAGGG GGCACCTGG GGCACCTGG AGCTCGGCT GTCTCAGTGC CCCTTGGGCG  
 CACCGGAGC GTACCGGAGC CTGCGGCTG GGTACCGGT GTTGGATGCC CCCTTGGAGC CCCTTGGGCT TCGGCGGCA CAGAGTCACT GGCACCTGGC  
 308 G P A N A M E G E P M R R H L O G N L G D P R A R A V E V P L G O

1101 CCGTGGCTG CCGTGGCTG AGTGGCGCTT CCGTGGCTG GCGCGCTG TACTCTGAG CGAATCTCC TTCTCTCTG ATGTGGTGA CAATCTCTT  
 GCGAGCTCA CCGAAGAGC TCACCGGCA GCGAAGAGC CCGCGGAGC ATGAGAGTCT GTTTAGAGG AGTAGAGAG TACACACTT GTTAAGGAGA  
 341 R V A R F L O C R F L F A G P N L L P E E I S F I S D V V H N S

1201 CCGGCTGAG CCGGCTGAGT CCGGCTGAGC CCGTGGCTG CCGTGGCTG AGCTCGGCT AACTTCAGCA GCTTGGAGT GAGGCTGAG GCGGCTGAG  
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 374 P A L G G T F P P A F W W P P G P P P T H F S S L L L E P R C Q Q P

1301 CCGTGGCTG CCGGCTGAGT CCGGCTGAGC CCGTGGCTG CCGTGGCTG AGCTCGGCT AACTTCAGCA GCTTGGAGT GAGGCTGAG GCGGCTGAG  
 GCGGCTGAG CCGGCTGAGT CCGGCTGAGC CCGTGGCTG CCGGCTGAGT TGAAGTCTG CCAACTGCA CCGGCTGAG CCGGCTGAGT  
 408 V A K P E G S P T A I L I G C L V A I I L L L L L I A L N L W R

1401 CCGTGGCTG CCGGCTGAGT CCGGCTGAGC CCGTGGCTG CCGTGGCTG AGCTCGGCT AACTTCAGCA GCTTGGAGT GAGGCTGAG GCGGCTGAG  
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 441 A H W R R L L E K A E R R V L R E L T V H L S V P G D T I L I N

1501 AACCGCTG GTCTTGAAG GCGAGCTGAGT CCGGCTGAGT CCGGCTGAGT TCGGAGTGG CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT  
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 474 H R P G P R E P P P T Q E P R P R G M P P H S A P C V P M G S A L L

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 508 L S H P A Y R L L A T Y A R P F R G P G P P T P A W A K P T T

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 TAAAGTGG CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT  
 574 I V T L Q G V T G G H T T A V P A L P P G A V G D G P P R V D P P R

1901 CATCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT  
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 608 S R L R P E E K L G E G Q P G E V H L C E V D S P Q D L V S L D P

2001 CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT  
 GCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT  
 641 P L N V R E G H P L L V A V E I L R P D A T E H A R M D P L K E V

2101 AAGTCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT CCGGCTGAGT  
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 674 E I S R L E D P E I I L L G V C V Q D D P L C N I T D V M E N G

Transmembrane Domain?

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Date           

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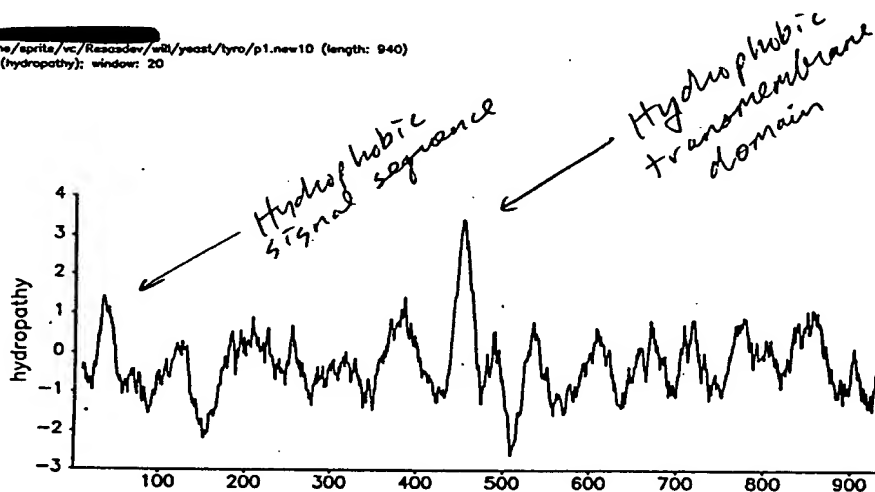
Date           

Will Bacon

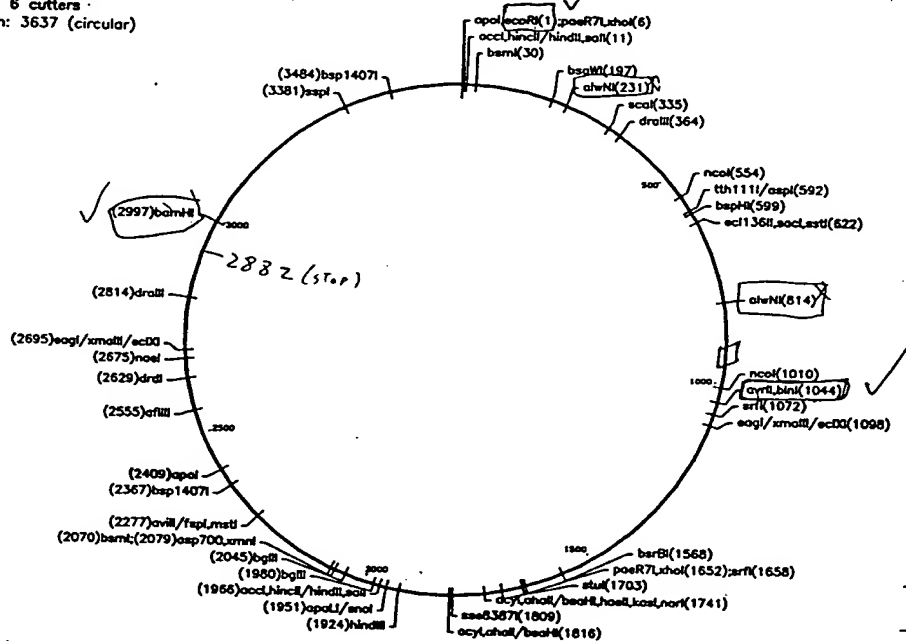


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/home/sprite/vc/Resasdev/will/yeast/tyro/p1.new10 (length: 940)  
lyte (hydropathy); window: 20



/home/sprite/vc/Resasdev/will/yeast/tyro/ss.new10  
sites: 6 cutters  
length: 3637 (circular)



Ultimately I will subclone an EcoRI/BamHI fragment into pRK5 for transfection into mammalian cells.

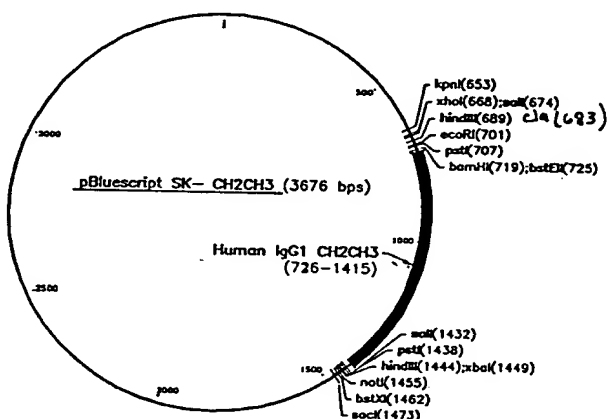
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Want to make ECD / IgG II fusion construct  
for Antibody production.  
70% sequence of vector from B. Bennett

## IgG Fusion Vector (For EC domains)

3676 (circular)  
human IgG1 heavy chain constant regions 2 & 3 = CH2CH3 in Bluescript SK minus  
-CH2CH3  
ps  
2 hitters in polylinker

bdb: [REDACTED]



/MolBio/bdb/ss.pBSSK-CH2CH3

des: 6 cutters

vch: 3676 (circular)

constructed by Brian Bennett 05.10.90

contains human IgG1 heavy chain constant regions 2 &amp; 3 = CH2CH3 in Bluescript SK minus

1 CACCTGACGC GCCCTGTAGC GCGCCATTAA CGCCCGCGGC TGTGGTGGTT ACGCCGACGC TGACCGCTAC ACTTGGCAGC GCGCTACGCG CCGCTCTCTT  
GTGGACTGCG CGGGACATCG CCGCGTAATT CGCGCGCGCC ACAACACCAA TCGCGCTGCG ACTGGCGATG TGAACGCTCG CGGGATCGCG GCGCAGGAAA

01 CCGCTTCTTC CTTCTCTTC TCGCCACCTT CCGCGGCTTT CCGCCCTCAAG CTCTAAATCG GGGGCTCTCT TTAGGCTTCC GATTTAGTCC TTTCAGGAC  
GCGAAGAAG GGAAGAAG ACCTGTGCAA CCGCGCGGAA GGGGCGATTC GAGATTAGC CCGCGAGGGA AATCCCAAGG CTAAATCAGC AAATCGCTCG

01 CTCGACCCCA AAAAAGTGA TTAGGCTGAT GTTCACCTGA GTGGGCGATC GCGCTGATAC ACCTTTTTC GCGCTTTCAG CTGCGAGTCC AGCTTCTTA  
GAGCTGGGCT TTTTGAATC AATCCCACTA CCAAGTGCAT CACCGGCTAG CCGGACTATC TGCCAAAAGG GCGGAAGTCA CAACCTCAGG TGCAAGAAAT

01 ATAGTGGACT CTCTTCCAA ACTGGAACAA CACTCAACCC TATCTGCTG TATCTTTTG ATTATAAGG GATTTTCCCG ATTTCCGCGT ATTTGTTAAA  
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01 AAATGAGCTG ATTTAACAA AATTAAACCG GAATTTTAC AAAATATTA CCGTTACAT TTCCATTGCG CATTCAGCTT GCGCACTGT TGCGAAGCG  
TTTACTGAC TAAATTTT TTAATTTGCG TTTTAAATG TTTTATAAT CCGAATCTTA AAGTAAGCG TTAAGTCCA GCGCTTGACA ACCCTTCCCG

01 GATCGGTCGC GCGCTCTTC CTATTACCG AGCTCGCGAA AGGGGATGT GCTGCAAGCG GATTAAGTGG GGTAAAGCCA GCGTTTCCCG AGTCAGGAG  
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Date

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[Signature]

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CTTTTCCTG CCAATCGAG AGCCAGGAG GTAGCAACA CTCTTCATC AACCGGCTC ACAATAGTGA GTACCAATAC CTTCTGAGC TACAGAGA  
 3201 TACTGTCATG CCATCCGTAA GATGCTTTC TGTGACTGT GAGTACTCAA CCAAGTCATT CTGAGAATAG TGTATGGGG CACCCAGTTC CTCTTGCCCG  
 ATCAGACTAC GTTAGGCATT CTAGCAAAAG ACACTGACCA CTCATGAGTT GCTTCAGTAA GACTCTTATC ACATACGCCG CTGGCTCAAC GAGAAGCGGC  
 3301 CCTCAATAC GGGATAATAC CCGCCACAT AGCAGAATT TAAAGTCTT CATCATGGA AAACGTTCTT CGGGCCGAAA ACTCTCAAGG ATCTTACCGC  
 CGCACTTAGG CCTATTATG CGCGGGTGA TGTCTTGAA ATTTTCACGA GTAGTAACCT TTTGCAAGAA GCGCCGCTT TGACACTTC TAGAATGGCG  
 3401 TTTTGAGATC CAGTTCATG TAACCCACT CTCATCTTCA GCATCTTTTA CTTTCACCAG CTTTCTGGG TGACCAAAA CAGGAAGGCA  
 ACAACTCTAG GTCAAGCTAC ATTGGCTGAC CAGTGGGTT GACTAGAAT CCTAGAAAAT GAAAGTGGTC GCAAGAGCC ACTCGTTTT CTCTTCCCT  
 3501 AAATCCCGCA AAAAGGGAA TAAGGGGAC AGCGAAATCT TGAATACTCA TACTCTCTT TTTTCAATAT TATTGAAGCA TTTATCAGG TTTATGCTC  
 TTTAGGGCT TTTTCCCTT ATTCCCGCT TGCCTTTACA ACTTATGAT ATGAGAAGCA AAAAGTATA ATAACTTCT TTTATGCTC  
 3601 ATGAGCGGAT ACATATTGA ATGTATTAG AAAATAAAC AAATAGGCT TCGGCGACA TTTCCCGGAA AACTGC  
 TACTCGCTA TGTATAACT TACATAAAT TTTTATTG TTTATCCCA AGCGGCTCT AAAAGGCTT TTTACG  
 length: 3676

Want to sequence 1 Hep5 + 1 Hep6 w/ GT10a + GT  
 to make sure that I have full length  
 Tyro 10 (as of now it is only complete  
 in the computer).

Kinased primers

Per rxn mixed

1 µl 10x buffer  
 1 µl polynucleotide Kinase  
 3 µl 8 <sup>32</sup>P ATP  
 2.42 µl 1:10 diluted primers  
 2.58 µl H<sub>2</sub>O  
 10

Inc 37°C 30'

Ran 1 mol sequencing as in Book # 17041  
 p. 87 → added stop buffer-mixed  
 Poured 2 sequencing gels

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sequencing gel recipe

24g urea

6.4ml 40% acrylamide

20.5ml H<sub>2</sub>O

5ml 10x TBE

50ml

Filtered Through 0.45µm Nylon

Added 400µl 10% APS → mixed

" 15µl TEMED → "

Poured → polymerized ~1hr RT

Pre-ran 65V ~ 30'

Denatured samples 100°C 2'

Loaded ~2µl each

Run wedge gel ~1hr 20' → transferred to

3mm paper → dried 80°C under vacuum

A/R'd RT 2hrs Then RT O/N.

Flat gel ran 65V ~ 3.5 hrs → transferred  
to 3mm paper → dried 80°C under  
vacuum ~ 30'

A/R'd RT O/N.

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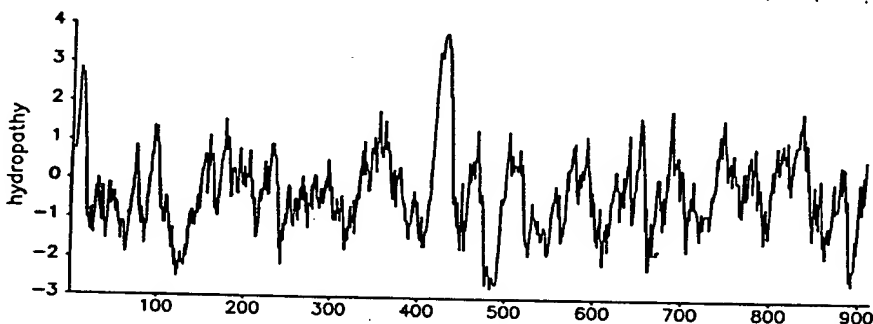
M. H. H. Brown



From Page No. 8

Developed O/N A/R's → read sequences of Tyro 10  
Only 5' ends of 15+6 were readable. (HPTK)

I will try cutting out complete coding  
sequence w/ EcoRI / BamHI & sequence  
entire clone if needed.



PREDICTED MOL. WT = 101,153

PREDICTED ISOELECTRIC POINT = 6.76 (REDUCED CYSTEINES)  
6.77 (OXIDIZED CYSTEINES)

Definite strongly hydrophobic signal sequence.

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